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SHORT SHEET: IEUBK MODEL BIOAVAILABILITY VARIABLE

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**U.S. ENVIRONMENTAL PROTECTION AGENCY
TECHNICAL REVIEW WORKGROUP FOR LEAD**

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IEUBK Model Bioavailability Variable

Introduction

Performance of the Integrated Exposure Uptake Biokinetic Model for Lead in Children (IEUBK) is a function of site-specific parameter input values. A site-specific determination of soil-borne lead bioavailability is, therefore, advantageous for improving predictiveness of the model. This short sheet discusses issues to consider and applicable methods for determining a site-specific bioavailability value for soil-borne lead.

The current default estimate for the bioavailability of soil/dust in the IEUBK model is 30 percent as an absolute value. This absorption fraction is partitioned into a non-saturable component (6 percent) and a saturable component (24 percent). Investigators (Casteel ; Henningsen *et al.*, 1998) have observed variable bioavailability across different soil/lead matrices, although the majority of samples are generally consistent with the IEUBK default value. Soil particle size (for soils sieved to <250 µm or 60-mesh), mineralogy, and lead speciation are among the factors that influence bioavailability (Steele *et al.*, 1990).

In Vitro techniques, such as the physiologically-based extraction test (PBET - Ruby *et al.*, 1996), have been developed as a means of capturing the impact of the soil/lead matrix on bioavailability. However, physico-chemical characteristics of the soil/lead matrix are not the sole determinants of the highly complex biological process of gastrointestinal absorption. In effect, solubility and bioavailability are not interchangeable terms. Until such time that fully validated *in vitro* techniques become generally accepted, the recommended approach to demonstrating site-specific bioavailability will need to be supported by an appropriate animal bioassay.

This short sheet reaffirms the provisions of the 1995 *Administrative Reform for Lead* that requires review of data that may set a precedent. Bioavailability data (other than from published studies using the juvenile swine model) that are intended for use in an EPA risk assessment using the IEUBK should be sent for review by the Office of Emergency and Remedial Response. This review not only promotes better science but also promotes sharing of information so that all EPA Regions can benefit from new information/analyses.

Definitions

As indicated in the Guidance Manual for the IEUBK Model, **bioavailability** refers to “the fraction of the total amount of material in contact with a body portal-of-entry (lung, gut, skin) that enters the blood.” Bioavailability is also described as absolute or relative (USEPA, 1994). **Absolute bioavailability** is the amount of a substance entering the blood via a particular route of exposure (*e.g.*, gastrointestinal) divided by the total amount administered (*e.g.*, soil lead ingested). **Relative bioavailability** is indexed by measuring the bioavailability of a particular substance relative to the bioavailability of a standardized reference material, such as soluble lead acetate.

It should be noted that the bioavailability input parameter in the IEUBK model is an absolute value, but it may be experimentally determined by relative means, provided that the absolute bioavailability of the “standardized reference material” is known. For the IEUBK model, soluble lead in water and food is estimated to have 50 percent absolute bioavailability. The model presumes that the relative bioavailability of lead in soil is 60 percent, thus producing an absolute bioavailability for soil lead of 30 percent (*i.e.*, 60% x 50% = 30%). It is acknowledged that this value has significant variability and uncertainty, but it is the estimate under which the IEUBK model was validated with comprehensive blood lead study results.

“**Bioaccessability**” is a term used in describing an event that relates to the absorption process. It generally refers to the fraction of administered substance that becomes solubilized in the gastrointestinal fluid. For the most part, solubility is a prerequisite of absorption, although small amounts of lead in particulate or suspended/emulsified form may be absorbed by pinocytosis. Moreover, it is not simply the **fraction** dissolved that determines bioavailability, but also the **rate** of dissolution, which has physiological and geochemical influences. In and of itself, bioaccessability is not a direct measure of the movement of a substance across a biological membrane (*i.e.*, absorption or bioavailability). The relationship of bioaccessability to bioavailability is ancillary and the former need not be known in order to measure the latter.

However, bioaccessability (*i.e.*, solubility) may serve as a surrogate for bioavailability if certain conditions are met (see *Methods and Issues to Consider when Determining Site-Specific Bioavailability of Soil-Borne Lead*).

As previously mentioned, lead absorption is believed to occur by both **active** and **passive** mechanisms. Although the precise subcellular processes involved in lead absorption are not entirely known, active/passive absorption processes (depending on dose) can impart a curvilinear shape to a graph of dose vs blood lead concentration. The potential impact of active and passive absorption processes on the determination of relative bioavailability is discussed in a latter section (*Methods and Issues to Consider when Determining Site-Specific Bioavailability of Soil-Borne Lead*).

When to Consider Adjustments in Bioavailability

As stated in the *Introduction*, the bioavailability of soil-borne lead is influenced by numerous characteristics of the soil-lead matrix. Particle size has been demonstrated to effect soil-lead bioavailability (Steele *et al.*, 1990). Although a strong quantitative relationship between particle size and bioavailability has not been established, an understanding of particle size distribution in a soil-lead source may provide qualitative information on the potential bioavailability of the source material. Perhaps more importantly, available data (Henningsen *et al.*, 1998) indicate that lead speciation can have a significant effect on bioavailability.

Currently, *in vivo* bioassays are the only way to quantitatively measure and adjust default bioavailability to fit site soils. However, validation studies are in progress which show promise for *in vitro* tests which may be correlated to the *in vivo* results. Such a test would have obvious and much needed advantages of speed, affordability, simplicity, and higher throughput. Until such tests are sufficiently validated with *in vivo* data, the use of *in vitro* bioaccessibility results are deemed by EPA to represent insufficient evidence for quantitative adjustment of bioavailability. The reason for this position is that small changes in *in vitro* assays, such as pH, time, temperature, volume, other solutes, and agitation regimes, can have relatively large impacts on results of lead solubility. Until validation is confirmed, the use of a simpler, faster, and cheaper lab benchtop test will not, in and of itself, be judged an adequate surrogate for measuring bioavailability.

Results of tests by EPA using animal models have shown a general pattern of relative bioavailability for certain lead salts. While lead speciation is not the sole factor influencing bioavailability, these patterns can, nonetheless, be used to compare a site's form of soil lead to explore differences in bioavailability relative to the defaults. If the lead speciation profile suggests a bioavailability estimate substantially different from the IEUBK model default, then the costs and benefits of performing supporting animal tests for now, and possibly of *in vitro* tests after validation, can be considered for quantitative

measures of bioavailability, and adjustments for a specific site. Furthermore, qualitative estimates of relative bioavailability can be made in the uncertainty section of a risk assessment. General patterns of relative bioavailability determined by EPA Region 8 studies of 20 soil lead samples (Henningsen *et al.*, 1998), compared to the default soil relative bioavailability of 60 percent, are shown as groups in the following table:

| Potentially Lower Bioavailability (RBA < 25%) | Intermediate Bioavailability (RBA = 25 % to 75%) | Potentially Higher Bioavailability (RBA > 75%) |
|---|---|--|
| Galena (PbS) Anglesite (PbSO ₄) Pb (M) Oxides Pb Fe (M) Sulfates Native Pb | Pb Oxide Pb Fe (M) Oxides Pb Phosphate Slags | Cerrusite (PbCO ₃) Pb Mn (M) Oxides |

Pb = lead, S = sulfur, M = metals, Fe = iron, Mn = manganese

Results of well-conducted blood lead studies can infer relatively low bioavailability of lead in soil. Such findings would not support a quantitative adjustment of bioavailability, but could assist in identifying soils for further study and/or support a qualitative adjustment in the risk characterization section of a risk assessment.

Methods and Issues to Consider When Determining Site-Specific Bioavailability of Soil-Borne Lead

Ethics aside, in a hypothetical setting the ideal method for making a bioavailability adjustment for soil-borne lead in the IEUBK model would be to dose a large group of young children with soil-borne lead and compare the area-under-the-concentration/time curve (AUC) with the AUC of the same or similar group which received an equal lead dose by intravenous administration. This is the conventional pharmacological and toxicological method for measuring *absolute* bioavailability. Realistically, issues of ethics, cost, and implementation are important determinants of study design. Consequently, an alternate approach is to measure soil-lead bioavailability *relative* to a "standardized reference material" (see *Definitions* section).

Determination of relative bioavailability needs to consider the experimental evidence suggesting that gastrointestinal lead absorption follows first-order saturation kinetics. An example is presented to illustrate that relative bioavailability, as estimated from experimental studies, can depend strongly on the response levels at which comparisons are made. The approach used to estimate relative bioavailability is to compare doses of lead (in different forms) that, upon ingestion by an experimental animal, produce equal levels of biological response (in this example, blood lead concentrations). The curves in the Figure illustrate relationships that may be fit to experimental data on the relationship between the ingested dose of lead and resulting blood lead measures. The two curves are of the Michaelis-Menten form



(Equation 1) with $v_{max} = 30$, $k_m = 1$ in the soluble lead relationship and $v_{max} = 10$, $k_m = 0.4$ for the soil lead relationship.

$$\text{Absorption rate} = \frac{v_{max} \times \text{dose}}{k_m + \text{dose}} \quad \text{Equation 1}$$

where

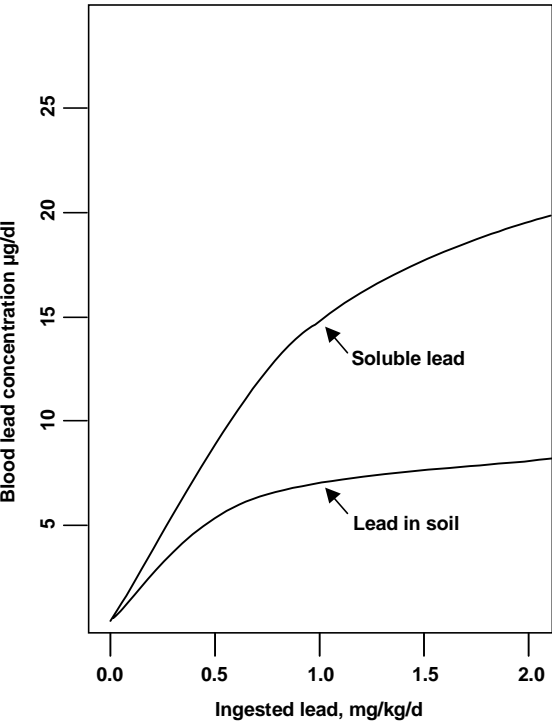
- v_{max} = maximum rate at which an enzyme can function.
- k_m = concentration of substrate that produces 50% maximum velocity of the enzyme.

This example is hypothetical, in that the curves shown are for purposes of illustration and are not intended to represent a specific data set. However, similar models, using Michaelis-Menten form equations, have been presented to EPA as models of bioavailability data from rodent studies conducted with soils from Superfund sites.

To estimate relative bioavailability in this example, a reference blood lead concentration is selected (5 µg/dL). The dose levels of soluble lead and lead in soil, respectively, at which this blood concentration is produced are then estimated. As illustrated in the Figure, a dose of 0.2 mg/kg/d of soluble lead is associated with a blood lead level of 5 µg/dL, while a dose of lead in soil of 0.4 mg/kg/d is required to achieve this same level. The relative bioavailability is estimated to be 0.5 or 50 percent based on the ratio of these doses (0.2/0.4).

However, in this example, where the soluble lead graph and the soil lead graph show different curvatures (specifically resulting from the different k_m values in the example), the estimated relative bioavailability depends on blood lead level at which the comparisons are made (see figure at right).

Hypothetical response curve for lead uptake study
Example showing different stages of response for soil lead and soluble lead



Note that at low doses the relative bioavailability of the two materials is similar, while at high doses the relative bioavailability of lead in soil is estimated to be low compared with soluble lead. A variety of different mechanistic factors may affect the bioavailability of lead administered at high doses. In experimental studies of bioavailability, substantial amounts of soil may be administered to the experimental animals, and the presence of these high quantities of soil in the diet may affect the bioavailability of lead. Such effects may be due to alterations to the chemical environment of the GI tract.

| Blood lead concentration for calculating relative bioavailability (µg/dL) | Dose of soluble lead to achieve this concentration (mg/kg/day) | Dose of soil lead to achieve this concentration (mg/kg/day) | Relative bioavailability |
|---|--|---|--------------------------|
| 1.00 | 0.03 | 0.04 | 0.78 |
| 2.00 | 0.07 | 0.10 | 0.71 |
| 3.00 | 0.11 | 0.17 | 0.65 |
| 4.00 | 0.15 | 0.27 | 0.58 |
| 5.00 | 0.20 | 0.40 | 0.50 |
| 6.00 | 0.25 | 0.60 | 0.42 |
| 7.00 | 0.30 | 0.93 | 0.33 |
| 8.00 | 0.36 | 1.60 | 0.23 |



For example, the presence of substantial quantities of soil may provide additional binding sites for lead, reducing the likelihood that any lead which becomes solubilized will remain in solution and be absorbed. Due to the potential for high doses of either soil or lead itself to affect (reduce) absorption, experimental bioavailability studies need to be performed at low enough doses to provide a reasonable comparison with the quantities of soil and lead that humans are likely to ingest. Where experimental limitations necessitate that the quantities of soil or lead administered substantially exceed the expected human doses (on a body weight basis), it should be recognized that an extrapolation to lower doses may be appropriate. This extrapolation step may take the form of an explicit mathematical treatment of the data (and as such would need to address the uncertainty in the predictions at low dose) or it may involve a more qualitative demonstration that under the particular experimental conditions utilized, the estimated bioavailability is not highly sensitive to the lead dosage used for comparison.

Site Soil Homogeneity for Sample Collection and Preparation

Soil samples that are tested for *in vivo* bioavailability or *in vitro* bioaccessibility should be obtained from areas that are reasonably similar (*i.e.*, similar geophysical and chemical properties of lead in soil). The top 2 inches of surface soil from residential yards should be representatively sampled and composited for testing. It is critical to sieve soil samples to <250 μm (60 mesh) to more closely represent the size of soil particles that would be expected to adhere to children's hands. An extremely useful tool for geophysical-chemical characterization of lead in soil is the electron microprobe (Medlin, 1997). Soil samples which are characterized or tested for bioavailability must retain their integrity, including chain of custody documentation, and proper mixing that provides a uniform subsample without physically degrading the soil particles.

Appropriate Animal Model

Because of the difficulties in gathering data on oral absorption of lead in children, there is no validated **absolute model** for experimental uses in measuring bioavailability. Each candidate animal model is expected to respond uniquely to absolute lead absorption (*i.e.*, oral uptake *vs.* intravenous dosing), compared to children, because of differences in physiology, diet, behavior, and development. However, it is possible to use a similar mammalian gastrointestinal system to measure **relative** absorption in comparison to the uptake of a soluble lead reference material (*e.g.*, lead acetate). This is the concept underlying the juvenile swine model (Weis *et al.*, 1994) which has further advantages of permitting sequential blood sampling and responding to doses similar to those experienced by children. Further details on the appropriate design aspects of such studies can be obtained from Weis *et al.*, 1994; Casteel *et al.*, 1997; and Henningsen *et al.*, 1998.

Previous rodent studies have had limitations due to:

- (1) rapid development which often resulted in testing of sexually mature animals which may have lost some of their active transport uptake of lead;
- (2) inability to produce AUC blood lead results *vs.* time, due to rodents' small size which precludes repeat blood sampling;
- (3) necessity to dose rodents with exceptionally high doses of soil lead to generate elevations in blood lead. Such high doses would fall into the saturation portion of the dose-response curve for other animals and probably for children, making accurate extrapolations of bioavailability difficult, if not impossible;
- (4) delivery of soil lead to rodents in food *vs.* in a small amount of vehicle, due to practical matters of dosing by oral gavage. This prevents assessment of bioavailability in a partially fasted state and results in a highly variable dose (mg/kg-d body weight) over the term of exposure due to high growth rates in rodents; and
- (5) other confounders unique to rodents. Other animal models also have had their limitations in estimating quantitative bioavailability of lead in soil, and so the juvenile swine model used by EPA appears to be the most useful model.

Currently, the juvenile swine model (Weis *et al.*, 1994) design offers the strongest method to measure site-specific bioavailability, since it attempts to mimic childhood absorption and doses of lead in soil relative to soluble lead acetate. Critical to this or any future or alternative study is the need to test a representative soil lead sample which best reflects the geophysical and chemical nature of the lead in residential yards. Composite sampling of relatively homogeneous types of lead in surficial soil can produce an acceptable test sample. In the near future, promising *in vitro* models may be validated that correlates well with the *in vivo* swine model results. When approved by EPA, these validated models will have utility for screening soil and dust samples for relative bioavailability and can provide quantitative measures of bioaccessibility that can reasonably predict bioavailability of lead in soils with an acceptable amount of uncertainty.

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